31. (New) The method as claimed in claim 18 wherein the serum supplement and the nutrient medium are combined in a 1:10 ratio, respectively.

REMARKS

Reconsideration of the above-identified patent application in view of the amendment above and the remarks below is respectfully requested.

Claims 1-7 and 9 have been canceled herein. Claims 8, 10-11, 14-15, 18, 21-22 and 26 have been amended herein. New claim 31 has been added herein. Therefore, claims 8 and 10-31 are pending. Of these claims, claims 8-16, 18-23, 27 and 31 are under active consideration.

On page 2, third paragraph, of the outstanding Office Action, the Patent Office states the following:

> Applicant has provided a "List of Figures" (page 3, lines 19-21) but has filed no figure(s). In accord with MPEP 601.01(F), this application is being examined on the basis that the drawing is not considered essential for establishing a filing date of 2/09/01. Applicant is required to cancel the reference in the specification to the Figures. MPEP 601.01(g).

Applicant respectfully traverses the Patent Office's requirement above that "the reference in the specification to the Figures" be canceled. This is because (even assuming *arguendo* that the figure in question was not filed with the present specification) the present specification incorporates by reference the disclosure of U.S. Provisional Patent Application Serial No. 60/181,614. As evinced by the enclosed copy of the filing receipt from said provisional application, a drawing was included in said provisional application. Therefore, because said provisional application is

incorporated by reference into the present application, said drawing is also a part of the present application. A copy of the drawing in question is enclosed herewith for use in the present application.

The disclosure stands objected to for the following reason.

> The following misspellings have been noted:
>
> | | |
> |---|---|
> | --Perfusion-- | at page 4, line 25 |
> | --rapid-- | at page 4, line 29 |
> | --washing-- | at page 4, line 29 |
> | --as-- | at page 5, line 4 |
> | --from-- | at page 5, line 15 |
> | --of-- | at page 5, line 16 |
> | --monoclonal-- | at page 6, line 10 |
> | --antibody-- | at page 6, line 10 |
> | --IgG-- | at page 6, line 24 |
> | --concentration-- | at page 7, line 7 |
> | --washed-- | at page 7, line 10 |
> | --serum– | at page 7, line 12 |
> | --is-- | at page 9, line 4 |
>
> Appropriate correction is required.

In response to the foregoing objection, Applicant has amended the specification to correct the misspellings noted above. Accordingly, the foregoing objection has been overcome and should be withdrawn.

Claims 8-15, 21-22 and 26 stand rejected under 35 U.S.C. 112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." In support of the rejection, the Patent Office states the following:

> In claim 8, line 4 "The first compound" lacks antecedent basis. Recitation of "compound" in dependent claim 14, line 2 is confusing because it is not clear how this relates to components recite[d] in the steps of claim 8.

In claim 8, line 6 "the mixture" lacks antecedent basis.

In claims 14 and 21, line 2 of each, "column is" is confusing. Applicant may intend insertion of –which– after "column".

In claim 22, line 2 "the culture medium" lacks antecedent basis.

In claim 26, "the cell culture protein" lacks antecedent basis.

Without acquiescing in the propriety of the rejection, Applicant has amended the claims to overcome the foregoing rejection. Therefore, for at least this reason, the foregoing rejection should be withdrawn.

Claims 8-12, 14, 16, 18-19, 21, 23 and 27 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Aybay et al. (Jour. Immunol. Meth., 233, 77-81, 2000 cited as ref. AB on form 1449)." In support of the rejection, the Patent Office states the following:

> Aybay et al.'s article is a proper reference under 102(a) because it has a publication date of 1/13/00.
>
> Aybay et al. teach the depletion of IgG from FCS, via affinity chromatography on Protein-G Sepharose. The eluant (designated as "G-FCS") is used to culture cells producing monoclonal antibodies, which are then purified from the eluant culturing medium. Regarding dependent claims 10-11, note that the abstract (line 2) teaches that the depletion was accomplished in less than 80 minutes.
>
> Aybay et al.'s method merely differs from that claimed by virtue of mixing the IgG depleted FCS with culture media, after the affinity chromatography step, rather than prior thereto. However, the instant change in the order of steps is taken as obvious because the end result is the same in either case –i.e., a culture medium containing IgG depleted FCS is used to culture cells producing monoclonal antibodies. Applicant has shown no unexpected advantage in conducting the steps in the order recited over what is disclosed by Aybay et al. –e.g., applicant's affinity chromatography step is not conducted more rapidly.

Insofar as the foregoing rejection pertains to claim 9, the rejection is moot in view of Applicant's cancellation herein of claim 9. Insofar as the foregoing rejection pertains to claims 10-12, 14, 16, 18-19, 21, 23 and 27, Applicant respectfully traverses the foregoing rejection.

Claim 18, from which claims 19, 21, 23 and 27 depend, has been amended herein and now recites "[a] method for obtaining a purified cell culture product comprising:

(A) selecting a serum supplement and a nutrient medium suitable for cell culture;

(B) combining the serum supplement with the nutrient medium to form a mixture;

(C) subjecting the mixture to a chromatography step so as to remove a compound capable of interfering with the preparation of the cell culture product, the chromatography step comprising perfusion chromatography and providing an eluant, and

(D) obtaining the purified cell culture product from cells grown or maintained in the eluant."

Claim 18 is not rendered obvious over Aybay et al. for at least the reason that Aybay et al. does not teach or suggest a method comprising, among other things, the steps of (i) combining a serum supplement and a nutrient medium to form a mixture and (ii) subjecting **said mixture** to perfusion chromatography to remove from said mixture a compound capable of interfering with the preparation of a cell culture product. Instead, Aybay et al. discloses a method wherein a serum supplement is subjected to a **Sepharose**-based affinity chromatography column and, **thereafter**, the eluant is combined with a nutrient medium.

Notwithstanding the fact that the claim requires, among other things, that the chromatography step be performed once the mixture is formed whereas Aybay et al. teaches forming the mixture after the chromatography step is performed, the Patent Office is apparently contending that such a

difference in the order of steps is immaterial "because the end result is the same in either case." Applicant respectfully disagrees. Aybay et al. discloses that its method was used to treat **20 ml** of fetal calf serum in less than **80 minutes**. By contrast, as explained in the present specification, the method of the present invention can be used to treat **600-2400 ml (or even higher) of mixture per hour**. As can be seen, there is a several-fold increase in the present invention. Therefore, the present method provides a clear and unexpected increase in the rate at which treatment may take place.

Moreover, even though, as shown above, the present method does exhibit an unexpected advantage over the method of Aybay et al., Applicant respectfully disagrees that such an advantage need be shown by Applicant to establish patentability. Applicant respectfully submits that the Patent Office has improperly shifted the burden of proof to Applicant by apparently contending that any change in the order of steps of Aybay et al. is obvious unless Applicant can establish an unexpected advantage. Applicant respectfully submits that such an approach is not a proper approach for determining obviousness. The initial operative issue is whether the prior art teaches or suggests modifying the prior art to arrive at the claimed invention - not whether the claimed invention is better than the prior art. The Patent Office has provided no evidence that the prior art provides any motivation to combine a serum supplement with a nutrient medium and to subject the mixture to chromatography (let alone perfusion chromatography). In the absence of any such motivation, no further inquiry is necessary.

Claim 8, from which claims 10-12, 14 and 16 depend, is patentable over Aybay et al. for at least the same types of reasons given above for claim 18.

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

Claims 8-16, 18-23 and 27 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Zeng et al. (5,593,822)." In support of the rejection, the Patent Office states the following:

> Zeng et al., like Aybay et al. noted supra, teach a method of depleting IgG from serum via affinity chromatography on Protein G (col. 9, lines 5-15). They further teach use of the IgG depleted serum in culturing media for the purpose of culturing cells producing monoclonal antibodies, and they teach the purification of monoclonal antibodies from the culture media thus employed. Though the order of steps taught by Zeng et al. differs from applicant's, obviousness is stated according to the same rational set forth above for Aybay et al.
>
> Regarding claims 13 and 20, Zeng et al. teach throughout that either Protein A or G may be used for depleting IgG from serum (e.g. col. 3, lines 7, 12, 19, 29, 38, 49-51; col. 4, line 56.)
>
> With respect [to] claims 15 and 22, Zeng et al. teach (col. 9, lines 10-11) a step of sterilization following the step of affinity chromatography.
>
> Regarding claims 10-11, Zeng et al. teach (col. 9, lines 7-9) recycling through a column of Protein G "overnight". This is certainly less than 24 hours and taken to have also been less than 12 hours, since research scientists are noted for working long hours.

Insofar as the foregoing rejection pertains to claim 9, the rejection is moot in view of Applicant's cancellation herein of claim 9. Insofar as the foregoing rejection pertains to claims 8, 10-16, 18-23 and 27, Applicant respectfully traverses the foregoing rejection.

Claim 8, from which claims 10-16 depend, and claim 18, from which claims 19-23 and 27 depend, are patentable over Zeng et al. for at least the same types of reasons discussed in the previous rejection.

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

Claims 13, 15, 20, 22 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Aybay et al. as applied to claims 8-12, 14, 16, 18-19, 21, 23 and 27 above, and further in view of Zeng et al. (5,593,822)." In support of the rejection, the Patent Office states the following:

> Aybay et al. have been noted supra for teaching depletion of serum via affinity chromatography on Protein G. They do not teach affinity chromatography on Protein A.
>
> Zeng et al. teach a similar method of depletion IgG from serum via affinity chromatography on Protein G (col. 9, lines 5-15). Zeng et al. also teach throughout that either Protein A or G may be used for depleting IgG from serum (e.g. col. 3, lines 7, 12, 19, 29, 38, 49-51; col. 4, line 56). It thus would have been obvious to use Protein A in lieu of Protein G in conducting the affinity chromatography step of Aybay et al., provided one were willing to accept any limitations involved in such a substitution of reagents (Aybay et al. at page 78, para. Spanning cols. 1-2). One would have been willing to accept such limitations in cases where one desired to culture cells producing IgG monoclonal antibodies of a subclass other than IgG1.
>
> Aybay et al. do not mention purification of the eluant from the affinity chromatography column; however, such a step following affinity chromatography of a composition to be employed for cell culturing is conventional, as shown by Zeng et al. (col. 9, lines 10-11). Since one would have expected the affinity chromatography step of Aybay et al. to be as equally likely of introducing microbial contamination as such step of Zeng et al., it would have been obvious to have sterilized what Aybay et al. obtained as their eluant.

Applicant respectfully traverses the foregoing rejection. Claims 13 and 15 depend from claim 8, and claims 20 and 22 depend from claim 18. Claims 8 and 18 are patentable over Aybay et al. for at least the reasons given above. Zeng et al. fails to cure all of the deficiencies of Aybay et al.. Therefore, claims 13 and 15 are patentable over the combination of Aybay et al. and Zeng et al. based at least on their respective dependencies from claim 8, and claims 20 and 22 are patentable

over the combination of Aybay et al. and Zeng et al. based at least on their respective dependencies from claim 18.

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

Claim 31, which is readable on the elected species, has been added herein. No new matter is added by claim 31. Claim 31 depends from claim 18 and is patentable at least on the basis of its dependency.

In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is

required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

Kriegsman & Kriegsman

By: _____

Edward M. Kriegsman
Reg. No. 33,529
665 Franklin Street
Framingham, MA 01702
(508) 879-3500

Dated: July 21, 2003

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Fee Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on July 21, 2003

_____

Edward M. Kriegsman
Reg. No. 33,529
Dated: July 21, 2003

MARKED-UP AMENDED SPECIFICATION

The paragraph bridging pages 4 and 5 is amended as follows:

Here a novel methodology is described where bovine IgG may be very rapidly removed from serum preparations for use in cell culture using immunoaffinity columns in [Perusion] Perfusion Chromatography (Afeyan et al., 1991). Perfusion chromatography involves the flow of liquid through a non-compressible porous chromatographic particle (POROS® Media, PerSeptive Biosystems) with 6000-8000 A pores which transect the particle. These through pores allow very high flow rates and enable [rapdi] rapid loading, [wasing] washing cleaning and elution of the column. We have applied perfusion affinity-chromatography using the BioCAD® Workstation.

The first full paragraph on page 5 is amended as follows:

Development and modernization of the methodologies of purification and isolation of protein molecules are needed to keep pace with the state-of-the-art technologies for protein purification and peptide analyses. The methodology described above serves [ass] as a model for the rapid [pruification] purification of all other MHC class I and class II molecules. The speed of purification reduces the handling time of the serum preparation and ensures an improved cost effectiveness and quality. Such a technological advance is fundamental to a sophisticated study of the immune response to foreign antigens, self-tolerance and autoimmunity and to the development of peptide vaccines based on the use of MHC-restricted epitopes for anti-tumor and anti-viral immunotherapy.

The second full paragraph on page 5 is amended as follows:

Pure monoclonal antibodies, humanized antibodies and Fc-fusion (chimeric) proteins are finding increased use in therapeutics and biomedical research. One of the more commercially popular ways is to produce antibodies as cell culture supernatant followed by affinity purification.

Most antibody producing cells require serum-containing medium. This medium preparation has antibodies [form] from the serum component. The spent medium thus has the antibody [if] of interest and the previously present antibodies. The prior art technique of separating an antibody of interest from the previously present antibodies is expensive and technologically involved. However, using this invention, a medium free of antibodies could be generated for the culture of antibody producing cells in a cost efficient manner.

The first full paragraph on page 6 (after the heading Production and Purification of Monoclonal Antibodies) is amended as follows:

The above medium was used to grow the [monnocolongal antibbodie] monoclonal antibody producing hybridoma cell line ME1 (an anti-HLA-B27 mouse IgG1 monoclonal antibody (Ellis et al., 1982), LB3.1 (an anti-HLA-DR, mouse IgG2b monoclonal antibody (Gorga et al., 1986) and 4418 (an anti NKp44, mouse IgG1 monoclonal antibody). The LB3.1 monoclonal antibody was purified by running the cell culture supernatant on POROS® 20A (protein A coupled POROS® 20 medium) and the ME1 and 4418 monoclonal antibodies were purified by running the cell culture supernatant on POROS® 20G protein G coupled to POROS 20® medium used for IgG1 antibodies) column using a BioCAD™ Workstation for perfusion chromatography (PerSeptive Biosystems). Typically 1-2 liters of cell culture supernatant was filtered through 0.2 micron filter and run on a POROS® 20A or a POROS® 20G column. The column was washed with 5 column volume of 2% acetic acid. The eluted antibody was immediately neutralized with 1M Tris base and the column equilibrated with PBS.

The second full paragraph on page 6 (after the heading Production and Purification of a Fc fusion protein exemplified by NKp46-Ig) is amended as follows:

Transiently transfected COS cells with NK-46Ig gene construct were grown in the [IggG] IgG free medium. The supernatant from these cells was run on POROS® 20G column as above and the pure NKp46-Ig fusion protein purified.

The paragraph bridging pages 6 and 7 (after the heading Preparation of Immunoaffinity Columns for removal of serum proteins from serum containing media prior to preparation of cell culture products) is amended as follows:

Typically 10-20 mg of the purified monoclonal antibody in PBS was coupled to one ml of POROS® 20 AL medium (POROS® 20 medium activated with the aldehyde group)(PE Biosystems). To about 5-10 mg/ml of antibody in PBS was added ½ volume of High Salt Buffer Solution (1.5 M sodium sulfate in 100 mM sodium phosphate 7.4). This was made 5-10 mg/ml in NaCNBH3 (Sigma). To this was added the appropriate amount of POROS® 20 AL (generally slightly more than the desired column volume) and the solution was made to 0.9-1.1 M in Na2SO4 by the addition of High Salt Buffer Solution. The final [conentraitoni] concentration of the antibody was between 1-2 mg/ml. The reaction was carried out overnight by gentle shaking. The media was filtered in a 10-20 m sintered glass funnel and resuspended in 50-100 ml of Capping Buffer (5 g/l NaCNBH3 in 0.2 M Tris, pH 7.2) for about one hour. The media was then [wased] washed with PBS and packed in a column. Columns ranging from 4.4 ml (100x7.5 mm) to 13.25 ml (300x7.5 mm) PEEK (polyetheretherketone) columns (Alltech) were packed under the conditions specified by the manufacturer. A pre-clearing column using normal mouse [seru8m] serum (NMS) was also prepared and used to remove proteins that adhered non-specifically to IgG.

The first paragraph on page 9 (after the heading Table 2) is amended as follows:

Pooled sequencing of HLA-B2705 eluted peptides

The yield in pmol of amino acid residues in each sequencing cycle of Edman degradation [si] is shown. The yields for the expected motif vix. arginine at position 2 and tyrosine, phenylalanine or leucine at position 9 are shown in bold.

MARKED-UP AMENDED CLAIMS 8, 10-11, 14-15, 18, 21-22 AND 26

8. (Amended) A method of preparing a culture medium containing serum[,] suitable for production in cells of a first protein in a class of proteins, the culture medium being deficient in a second protein in a related class, the second protein normally present in the serum and capable of interfering with the purification of the first [compound;]protein, the steps of the method comprising[;]:

(A) selecting [the] a culture medium containing serum;

(B) subjecting the [mixture] culture medium containing serum to an affinity chromatography step so as to provide a flow through, the flow through being deficient in the interfering second protein, wherein said affinity chromatography step is perfusion chromatography; and

(C) utilizing the flow through as a culture medium containing serum deficient in said second protein for production of the first protein by cells.

10. (Amended) A method according to claim [9]8, wherein step (b) further comprises completing the affinity chromatography step within 24 hours.

11. (Amended) A method according to claim [9]8, wherein step (b) further comprises completing the affinity chromatography step within 12 hours.

14. (Amended) A method according to claim 8, wherein the chromatography step includes [a chromatography column is] subjecting the culture medium containing serum to a perfusion chromatography column having a [compound] second protein binding ligand attached thereto.

15. (Amended) A method according to claim 8, wherein step (c) further comprises, the step of sterilizing the culture medium containing serum deficient in said second protein.

18. (Amended) A method for obtaining a purified cell culture product[;] comprising:

(A) selecting a serum supplement and a nutrient medium suitable for cell culture;

(B) combining the serum supplement with the nutrient medium to form a mixture;

(C) subjecting the mixture to a chromatography step so as to remove a compound capable of interfering with the preparation of the cell culture product, the chromatography step comprising perfusion chromatography and providing an eluant, and

(D) obtaining the purified cell culture product from cells grown or maintained in the eluant.

21. (Amended) A method according to claim 18, wherein the chromatography step includes [a chromatography column is] subjecting the mixture to a perfusion chromatography column having a compound binding ligand attached thereto.

22. (Amended) A method according to claim 18, wherein step (c) further comprises[,] the step of sterilizing the [culture medium] eluant.

26. (Amended) A method according to claim 18, wherein the cell culture [protein] product is a growth factor.

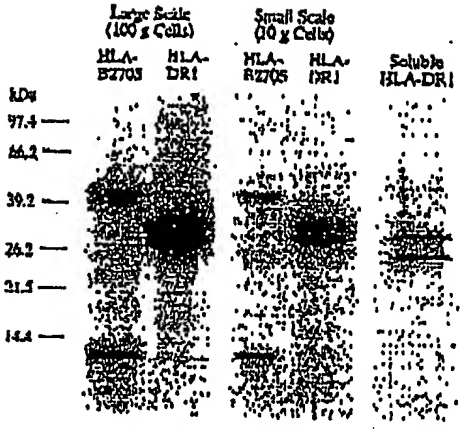

Fig. 1. SDS-PAGE analysis of purified HLA-B2705 and HLA-DR1 protein molecules from 100 g cells (large scale) and 10 g cells (small scale) and from cell supernatant of soluble HLA-DR1 producing cells. The gel was stained with Coomassie blue.